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Spumiform capillary basement membrane swelling: a new type of microvascular degeneration in senescent hamster

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ABSTRACT

Brain microvasculature plays a critical role in the regulation of homeostasis of neural tissues. The present study focuses on characteristic microvascular basement membrane (bm) aberrations in the midbrain periaqueductal gray matter (PAG) and their relation to aging. The PAG can be considered a caudal extension of the limbic system and is a key structure in the regulation of a myriad of autonomic and motor control functions. In an ultrastructural study, morphologic changes in mesencephalic PAG capillaries were assessed in aged and young hamster and compared with those in caudal brainstem areas. Bm aberrations were studied in 1200 capillaries ($n = 600$ young hamsters; $n = 600$ aged hamsters). A new, never reported variant of bm degeneration was found that presented itself as foamy-like structures accumulating within the lamina densa of notably PAG capillaries. We classified these foamy structures as 'spumiform basement membrane degenerations' (sbmd) in which we could distinguish 4 stages depending on the size and intramembranous localization, ranging from split bm (stage I), intermediate stages II and III, to extensive stage IV, affecting almost the complete capillary bm outline. In the PAG of senescent animals various stages of sbmd were observed in 92 ± 3% of all capillaries. Stage II was most prominently present (59%), followed by stage III (20%), and stage IV (13%). These bm aberrations were clearly age-dependent because in young animals, only 5% of the PAG capillaries showed characteristics of sbmd. For comparison, in the pontine reticular formation at the PAG-level, 41% of the capillaries showed a form of sbmd, but these defects were significantly less severe (stages I–II, 98%), and caudal brainstem structures displayed no sbmd at all. In addition to sbmd, diffuse endothelial changes, disrupted tight junctions, thickening of the bm, pericyte degeneration, and gliosis were observed in PAG capillaries. It is hypothesized that selective bm permeability of PAG capillaries results in a sequence of bm damage events that start with split bm, gradually changing into more and more extensive sbmd accumulations that eventually almost completely surround the capillary. Progressive sbmd in PAG capillaries might lead to a loss of blood–brain barrier function and consequently to impairment of autonomic and motor control functions exerted by the PAG.

1. Introduction

Most studies on aging of the vascular and microvascular condition in the mammalian brain focused on cerebral cortical and hippocampal regions, often in relation to neurodegenerative diseases and a compromised cognitive status (De Jong et al., 1999; de la Torre and Aliev, 2005; de la Torre; 2000, 2010a,b; Farkas and Luiten, 2001; Kalaria, 2003; Miller et al., 2007; Shah and Mooradian, 1997; Zlokovic, 2011). To our knowledge, no specific data are available from aging studies on microvascular conditions in subcortical regions such as the midbrain periaqueductal gray matter (PAG), despite the crucial role of the PAG in the control of a myriad of autonomic and motor control functions such as: control and expression of pain, analgesia, fear, anxiety, vocalization, lordosis, and cardiovascular function (Behbehani, 1995; Linnman et al., 2012; Paxinos and Mai, 2004).

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Exploring the neural substrate serving reproduction, we recently demonstrated a prominent columnar organization of nuclear estrogen receptor alpha (ER-α) immunoreactive neurons in the PAG projecting to the caudal brainstem of the female golden hamster, including nucleus retroambiguus, nucleus pararetroambiguus (NPRA) and commissural nucleus of the solitary tract (NTScom) (Gerrits et al., 2009b). The NPRA and the NTScom are part of a brainstem circuit comprising several interrelated nuclei that are subject to functional and structural plasticity and are intimately involved in the regulation of steroid hormone-dependent behaviors and their associated autonomic adaptations (Gerrits et al., 2008a,b, 2009b, 2010, 2012b).

As part of the ultrastructural study of steroid hormone responsive midbrain regions we included analysis of the microvascular condition in these steroid sensitive brainstem centers. No differences were observed between the microvascular changes occurring in the estrogen-receptive versus the estrogen-nonreceptive caudal brain stem areas of the female hamster brain. Despite commonly reported aging-associated neural and cerebrovascular degenerative changes including blood–brain barrier (BBB) impairment (Gerrits et al., 2010, 2012a), the animals displayed a reproductive behavioral repertoire comparable with young animals (Gerrits et al., 2009a; Veening et al., 2009). Furthermore, it was noticed that vascular degenerative aberrations like perivascular fibrosis which are commonly reported in the hippocampus of aging rats and other vertebrate species (De Jong et al., 1990; Farkas et al., 2001) were not seen in the brainstem of the aging hamster (Veening et al., 2009). Apparently, vascular impairments might be region-specific and not related to inhibitory components of estrous cycle-related behaviors.

Based on these and other observations we extended our interest to the aging effects on microvascular condition and structure in the midbrain PAG in view of the cardinal role of this brain region in autonomic regulation and its vulnerability during the aging process. Therefore, we analyzed the ultrastructure of the capillaries in the PAG in young (23 weeks) and aged (95 weeks) female hamsters and compared the PAG findings with estrogen-sensitive NPRA and NTScom, and the non–estrogen-sensitive medial tegmental field (mtf).

We unexpectedly discovered in the PAG capillaries a new kind of vascular aberration, as far as we are aware hitherto not described in the literature. Looking back into our previous investigations, we have observed and shown this aberration occasionally (Gerrits et al., 2010), but in the microvessels of the PAG this aberration turned out to occur most frequently. Especially in the PAG, successive stages in the development of this 'new' aberration can be observed. Because of the 'foamy' electron lucent character of the aberration, we have coined it as 'spumiform basement membrane degeneration' (sbmd). The regional differentiation of the occurrence of sbmd in the mesencephalic PAG and other brainstem areas forms the main content of our present study.

2. Methods

2.1. Animals

Four aged (95 ± 0.5 weeks) female golden hamsters (*Mesocricetus auratus*; cases H571, H574, H575, H576) weighing 130–140 g and 4 young (23 ± 0.5 weeks) female control hamsters (cases H547, H548, H552, H556), weighing 120–126 g were used for the present study. The experiments were performed on inbred animals obtained from Harlan (strain HsdHan: Aura; Harlan, UK Ltd.). All protocols, housing, and handling of the animals were in accordance with the ethical guidelines approved by the University Medical Center Groningen, University of Groningen (license number DEC 5142A). All necessary efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Housing and handling

All hamsters were housed separately in clear plastic cages in a 14/10-hour reversed light/dark cycle with food and water available ad libitum. Room temperature was maintained at 22 °C–24 °C and humidity at 50%–70%; wood shaving and straw were used as bedding materials. The animals were inspected daily for their general health condition and weighed once a week. Senescent animals were kept until 95–96 weeks, actually at the end of the female hamster lifespan (Gerrits et al., 2010, 2012b).

2.3. Tissue processing

2.3.1. Perfusion

After an overdose of nembutal (0.7 mL of 6% sodium pentobarbital intraperitoneally; Lundbeck Inc., Deerfield, IL, USA), the animals were transcardially perfused with 20 mL of heparinized phosphate buffer (0.1 M, pH 7.4), containing 0.4% sodium nitrite and 2% polyvinylpyrrolidone (molecular weight 40,000) at 37 °C, followed by 350 mL of fixative containing 0.05% glutaraldehyde, 4% paraformaldehyde, 0.2% picric acid, and 2% polyvinylpyrrolidone in 0.1 M phosphate buffer, pH 7.4, at room temperature. After perfusion, the brains were removed and postfixed for 1 hour in the same fixative at 4 °C.

2.3.2. Electron microscopy

Caudal brainstem and PAG tissue was cut on a vibratome in 60 μm transverse sections and collected in 0.01 M phosphate-buffered saline at 4 °C. Every other section was processed for a standard electron microscopy protocol: osmicated, dehydrated in a graded series of ethanol, and flat-embedded in Epon between dimethyldichlorosilane-coated glass slides. Samples of tissue containing the PAG and brainstem control regions were glued on Epon stubs. After blocking, the tissue was trimmed and cut into 1 μm semithin sections. Finally, 60 nm ultrathin sections from the selected structures were cut with a diamond knife for further electron microscopic analysis. At the ultrastructural level, 3 caudal brainstem structures (NPRA, NTScom, and mtf) and PAG were studied in detail. All microvascular and surrounding profiles were photographed at magnification × 10,000–20,000 using a Philips CM 100 electron microscope (Philips, Eindhoven, The Netherlands).

2.4. Control tissue

Brain tissue (PAG, NPRA, NTScom, and mtf) obtained from the young animals was processed exactly in the same way as the aged animals and served to control for possible aberrations as a result of tissue processing, and as a comparison group for aging-associated basement membrane (bm) degeneration. Tissue obtained from pontine reticular formation (prf) at the level ventrolateral to caudal PAG served as internal animal control (Fig. 1).

2.5. Photomicrography

The location of PAG, prf, NPRA, NTScom, and mtf were determined using a Zeiss Axioplan light microscope (Carl Zeiss Benelux, Trapezium 300, Sliedrecht, The Netherlands) at magnification × 10 (Fig. 1). Representative sections were photographed using a Leica DC500 digital camera and a Leica DM4000B photomicroscope connected to a Leica Q550IW computer and QWIN software (Leica Microsystems, Rijswijk, The Netherlands). Drawings of the sections were made using Adobe Illustrator 8.0 software (Adobe Systems, Mountain View, CA, USA).

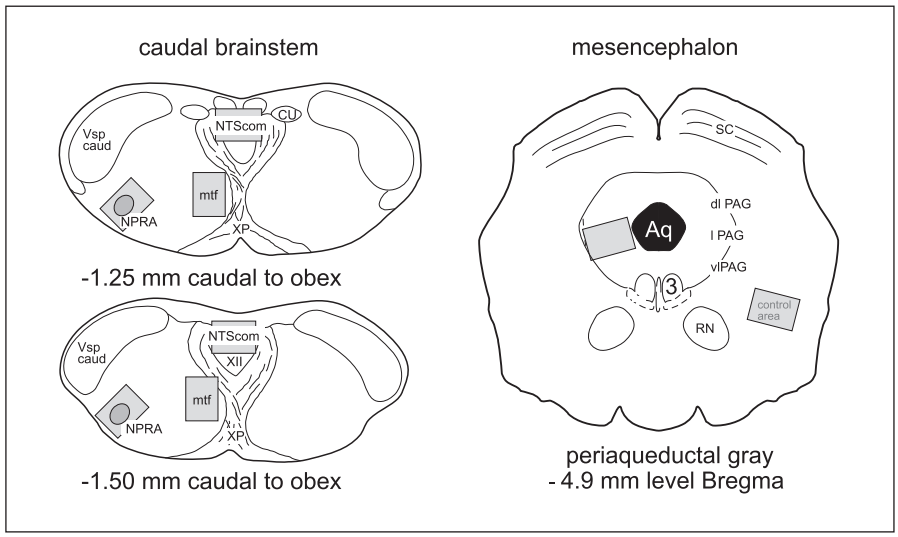

Fig. 1. Schematic overview of the 5 brainstem locations selected for capillary analysis. Caudal brainstem including nucleus pararetroambiguus (NPRA), commissural nucleus of the solitary tract (NTScom), and medial tegmental field (mtf). Mesencephalon with periaqueductal gray matter (PAG) and pontine tegmental field (prf, control area). Abbreviations: Aq, Sylvian aqueduct; CU, cuneate nucleus; e, endothelium; RN, red nucleus; sc, superior colliculus; Vspcaud, caudal spinal trigeminal complex; XP, decussation pyramidal tract; XII, hypoglossal nucleus; 3, ocular motor nucleus.

2.6. Quantitative analysis

Photomicrographs of capillaries were collected at random from thin sections of PAG, prf, NPRA, NTScom, and mtf, respectively. A total of 1200 capillaries were studied as described previously (Gerrits et al., 2010); 600 capillaries in 4 young hamsters and 600 capillaries in 4 aged hamsters. Per area, 30 capillaries per animal were studied. Basement membrane degeneration was classified (double blind) into 4 stages ranging from vacuolization (split bm [sbm], stage I) to extensive sbmd, surrounding almost the complete capillary bm outline (stage IV); see Fig. 2. Differences in the incidence of vascular abnormalities between the groups aged and young animals, and the rostral versus the caudal brainstem areas (caudal: NPRA, NTScom, mtf, and rostral: PAG and prf) were tested with a 2-tailed 2 sample *t* test assuming unequal variance between groups. To control for an inflated type I error resulting from 10 *t* tests we applied Bonferroni correction, which is very conservative in the scenario of 10 comparisons. In aged animals, location effects were tested with a single factor analysis of variance with location as independent variable and incidence of abnormalities as dependent variable.

3. Results

Based on our previous ultrastructural studies on capillary aging in the hamster (Gerrits et al., 2010) and concomitant neurodegenerative changes (Gerrits et al., 2012b), and the observations of extensive mutual relationships of specific bm aberrations, we describe a new, so far unreported, variant of bm degeneration. We named this new form of bm degeneration 'spumiform basement membrane degeneration,' because of its unique characteristic 'foamy-like' manifestation of electron lucent vacuoles within the confines of capillary bm and pericytic bm. The presence and classification of sbmd's was assessed in a total number of 1200 capillaries ($n = 600$ for young animals; $n = 600$ for aged animals) in mesencephalic PAG and pontine reticular formation, and caudal brainstem.

3.1. Classification of sbmd

Sbmd was classified in 4 stages (Fig. 2). Stage I was characterized by small, elongated and virtually empty splits (vacuoles) within the

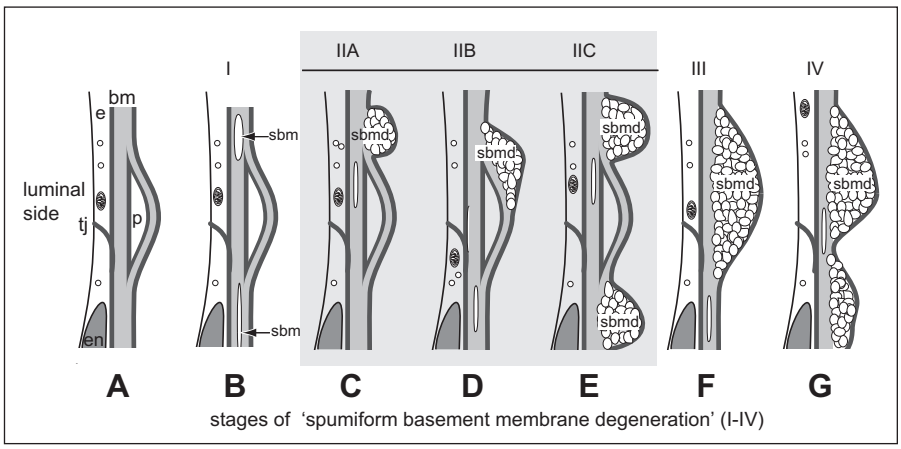

Fig. 2. Schematic representation of the stages (A–G) of spumiform basement membrane degeneration (sbmd). The gray boxed area shows 3 subdivisions of stage II (IIA–IIC). Note that split basement membrane (sbm) can be found in combination with all other manifestations. Abbreviations: bm, basement membrane; e, endothelial cytoplasm; en, endothelial nucleus; l, luminal side; m, mitochondrion; n, nucleus; p, pericyte; sbm, split basement membrane; tj, tight junction.

bm (sbm). These vacuolar splits vary in size and are surrounded by a thin electron dense membrane (Fig. 2B, Fig. 3B and H). Single sbm's were observed in combination with all other manifestations in the 3 later stages of the sbmd (Fig. 3H). Group II stages of spumiform degeneration have all in common that the split bm are filled with various quantities of foamy-like vesicles. For the sake of clarity, stage II was subdivided into categories IIA, IIB, and IIC. Stage IIA describes the condition in which translucent vacuoles within the bm were present on a single location. In the earliest phases of this stage membrane-like structures start to develop around the

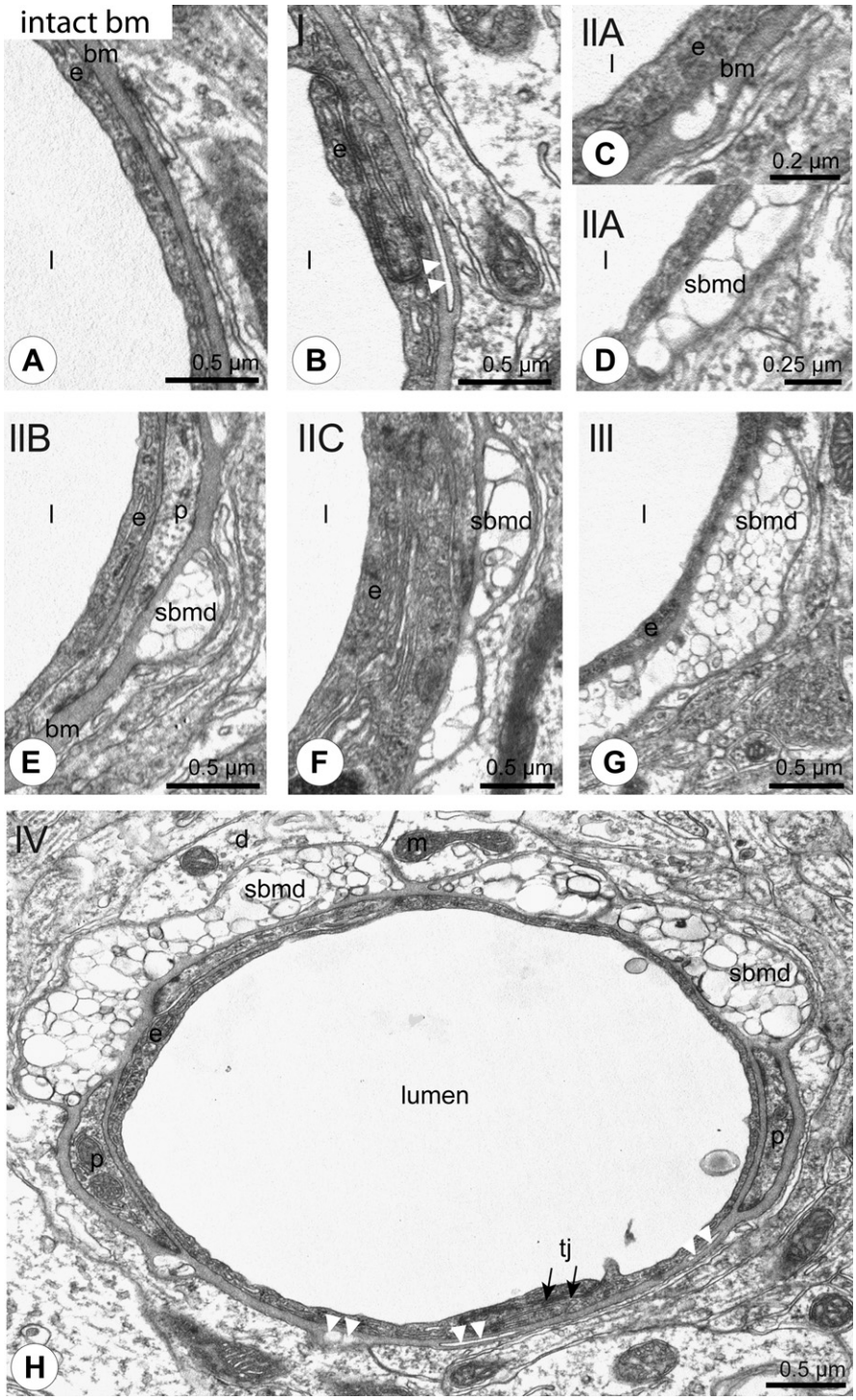

Fig. 3. Electron microscopic photomicrographs showing the different extensions of spumiform basement membrane (bm) degeneration (sbmd) in the periaqueductal gray matter (PAG) as presented schematically by Fig. 2A–G. (A) Intact bm composed of 3 laminae: lamina rara interna, lamina densa, and lamina rara externa. (B) Dilated bm in lamina densa surrounded by a thin membrane (white arrowheads). (C and D) Stage IIB. Split lamina densa with early neurodegenerative changes stages of formation of sbmd. Note the translucent appearance of the content of the split bm (sbm). (E) Solitary sbmd at the abluminal side of pericytic process. (F) Stage IIC. Two small isolated smbd's in close proximity, small remnants of the lamina rara interna, and lamina densa can be observed. (G) Extensive form of sbmd. Note the characteristic morphology of the translucent spumiform degeneration product. (H) Extensive form of sbmd covering approximately half of the capillary. Also at this stage of sbmd different forms of sbm can be observed (white arrowheads). Abbreviations: d, dendrite; e, endothelial cytoplasm; m, mitochondrion; p, pericyte; tj, tight junction.

vesicles (Fig. 3C) and the number of foamy vesicles progressively increases (Fig. 2C, Fig. 3C and D). Stage IIB manifests as small sbmd's with membranes at the abluminal side of the pericyte bm (Fig. 2D, Fig. 3E). In stage IIC multiple small, spatially separated sbmd's could be discerned around the capillary (Fig. 2E, Fig. 3F). Stage III is an extensive form of sbmd covering at least a quarter of the capillary bm (Fig. 2F, Fig. 3G). In stage IV pronounced sbmd was present, affecting more than half of the capillary bm outline (Fig. 2G, Fig. 3H).

3.2. Split bm and vacuolization in midbrain and caudal brainstem

3.2.1. Split bm in mesencephalon and caudal brainstem

Capillary bm splitting and vacuolization were common features of the aging midbrain. Sbm in aged animals was found in 78 ± 4 % (± 1 SD) of the bm of all screened PAG capillaries, and in 67 ± 4% of the prf capillaries (Fig. 4A). Sbm might be surrounded by a thin electron dense membrane but this was not necessarily always the case. The content of the vacuoles was typically translucent (Fig. 3B) in contrast to the thin cytoplasmic fragments of the pericytes containing electron-dense cell components (Fig. 3E; Fig. 5A). Capillaries in NPRA, NTScom, and mtf of the same senescent animals showed significantly less sbm (30 ± 5%; $t(11) = 14.4$; $p < 0.001$) than those in PAG and prf (Fig. 4A). Compared with the old animals, the presence of sbm in young animals was significantly lower ($t(21) = 8.26$; $p < 0.001$; Fig. 4A).

3.3. Sbmd in mesencephalon and caudal brainstem

3.3.1. Sbmd in mesencephalon

The analysis of capillaries in the PAG of old animals revealed that various forms of sbmd were present in 92 ± 3% of all capillaries (Fig. 4B). Stage II(A–C) of sbmd was most frequently present followed by stage III and stage IV, respectively (Fig. 4C left panel). In PAG of young animals only 5 ± 2% of all capillaries displayed sbmd (Fig. 4B), mainly of category IIA. In the pontine reticular formation, as an internal control area of old hamsters, sbmd was significantly lower than in PAG ($t(5) = 15.4$; $p < 0.001$): here only 42 ± 6% of all capillaries showed sbmd, mainly in the less prominent categories I and II (Fig. 4B and C) and incidentally as stage III. No spumiform aberrations were observed in prf of the young hamsters. Sbmd was also found accumulated in the bm abluminal to the endothelial and pericytic nuclei (Fig. 5B and C) Furthermore, extensive sbmd was also observed adjacent to and covering the pericytic cytoplasm (Fig. 5D).

In 2 rare cases collagen fibrils displaying characteristic periodicity were found intermingled with sbmd, similar to what has been described in the rat brain by (De Jong et al., 1990; Farkas et al., 2001), see Fig. 6A and B.

3.3.2. Sbmd in caudal brainstem

With respect to the development of sbmd, the most striking regional differences were found between PAG capillaries and caudal brainstem capillaries. The capillaries in NPRA, NTScom, and mtf of the same senescent animals showed significantly less sbmd (6 ± 3%; $t(7) = 6.2$; $p < 0.001$) than those in PAG and prf (Fig. 4B). Sbmd was never observed in NPRA, NTScom, and mtf of the young animals.

3.4. Generalized (peri)vascular aberrations

The present study focuses on splitting of the capillary bm and consequently the occurrence of the newly found sbmd. However, it has to be emphasized that in addition to these specific bm aberrations, other diffuse (peri)vascular and neurodegenerative changes were observed. In respect to capillary aberrations, these varied from endothelial changes, disrupted or widened tight junctions, thickening of the bm and pericyte degeneration to perivascular gliosis, comparable with the neurodegenerative changes reported previously (Gerrits et al., 2010, 2012a; Fig. 7A and C). Furthermore, increased amounts of neuronal intracytoplasmic lipofuscin (Fig. 7D), various forms of abnormal (giant) mitochondria, degenerated myelin accumulations, age-related bodies, and perivascular gliosis were found.

4. Discussion

The hamster is a very suitable mammalian model to study tumor biology, reproductive behavior, hibernation, neurodegeneration (present studies), and its underlying neuronal mechanisms. Its estrous cycle has an invariant duration of 4 days and remains highly regular even during the senescent phase of life. The estrous cycle affects not only reproductive behavior, but also feeding and drinking behavior (Attah and Besch, 1977; Blaustein and Wade, 1977;

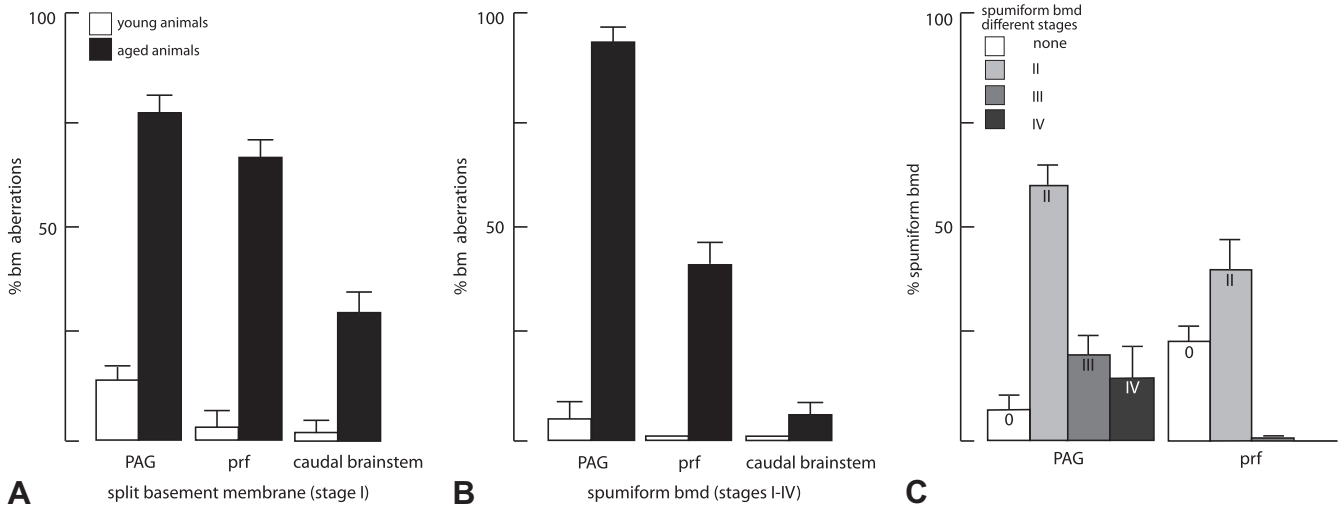

Fig. 4. Histograms showing the percentage of capillaries with split basement membrane and spumiform basement membrane degeneration. (A) Split basement membrane (sbm; stage I) and (B) spumiform basement membrane degeneration (sbmd; stages none–IV) in caudal brainstem, periaqueductal gray matter (PAG), and pontine reticular formation (prf) in young and aged hamsters. (C) Percentages of the 4 different sbmd stages per 30 capillaries; data shown as percentages ± 1 SD.

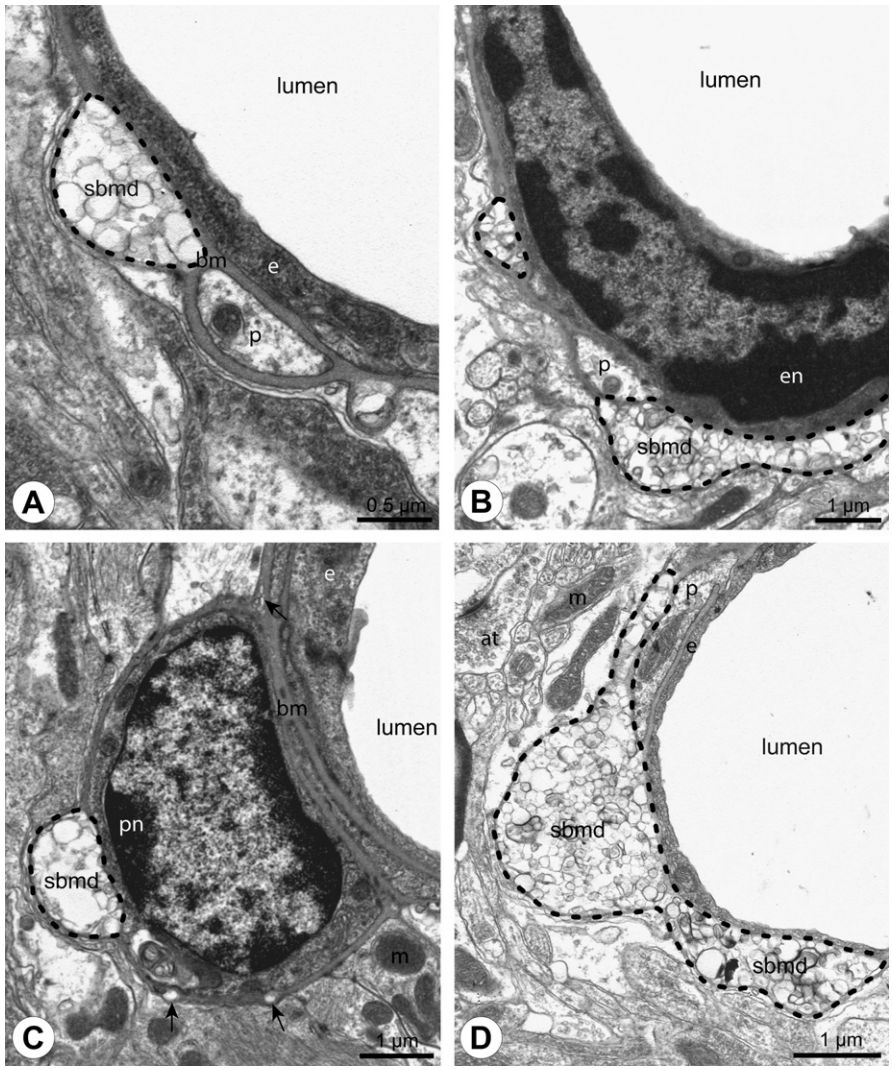

Fig. 5. Electron microscopic photomicrographs showing various forms of spumiform basement membrane (bm) degeneration (sbmd) in periaqueductal gray matter (PAG). (A) The content of sbmd is typically translucent in contrast to small peripheral parts of pericytic cytoplasm, containing electron dense cell components. (B) Sbmd at the abluminal side of an endothelial nucleus. (C) Sbmd at the abluminal side of a pericytic nucleus (pn). Arrows point to small bm splits. (D) Extensive sbmd present in endothelial bm and gradually spreading over peripheral pericytic cytoplasm. Stippled lines in (B) and (D) mark sbmd. Abbreviations: e, endothelial cytoplasm; en, endothelial nucleus; m, mitochondrion; p, pericyte.

Csakvari et al., 2007; Danielsen and Buggy, 1980; Findlay et al., 1978; Jennings, 1974), anxiety (McCormick et al., 2008; Mora et al., 1996), and pain responsivity (Frye et al., 1993; Ryan and Maier, 1988). Furthermore, there is increasing evidence that estrogen can mediate autonomic and cardiovascular adjustments and adaptive homeostatic control mechanisms (Orsini, 1961; Saleh and Connell, 2003; Saleh et al., 2000, 2005).

In the present ultrastructural study of the female hamster brain we compared the microvascular changes in fine structure during aging in the mesencephalic PAG with those of other, more caudal brainstem areas. The microvascular bm is a crucial part of the BBB and the presence of deranged parts of the BBB in aging animals and humans might directly or indirectly lead to impairment of neuronal functioning (Ballabh et al., 2004; Mooradian, 1988; Shah and Mooradian, 1997).

Concerning the bm itself, various common elements of bm aberrations were observed, in agreement with earlier studies (Gerrits et al., 2010). Capillaries were found in which the bm was virtually regular, but displayed a gradual thickening around the vessel together with thin membrane extensions protruding into the surrounding neuronal tissue. Further, irregular bm thickenings were observed, similar to those described in the rat and other mammalian species including man (De Jong et al., 1990; Farkas and Luiten, 2001; Farkas et al., 2001). These irregular forms of bm thickenings were often present in combination with protrusions extending into the deeper cell layers of the neuropil (Gerrits et al., 2010).

Apart from these capillary bm aberrations, the adjoining neural tissue showed nearly similar degenerative changes in all investigated aged brain areas. These changes ranged from increased cytoplasmic lipofuscin, abnormal and giant mitochondria, various forms of myelin degeneration, and considerable numbers of age-related bodies, to perivascular gliosis (Brunk and Terman, 2002; Farkas and Luiten, 2001; Farkas et al., 2006; Gerrits, 2009b; Gray and Woulfe, 2005; Jung et al., 2007; Terman and Brunk, 1998; Veening et al., 2009).

4.1. Sbmd

A striking novel finding was that in addition to the above-mentioned bm changes, a new form of bm degeneration was found. Our present study for the first time provides ultrastructural evidence that age-related microvascular bm pathology in the

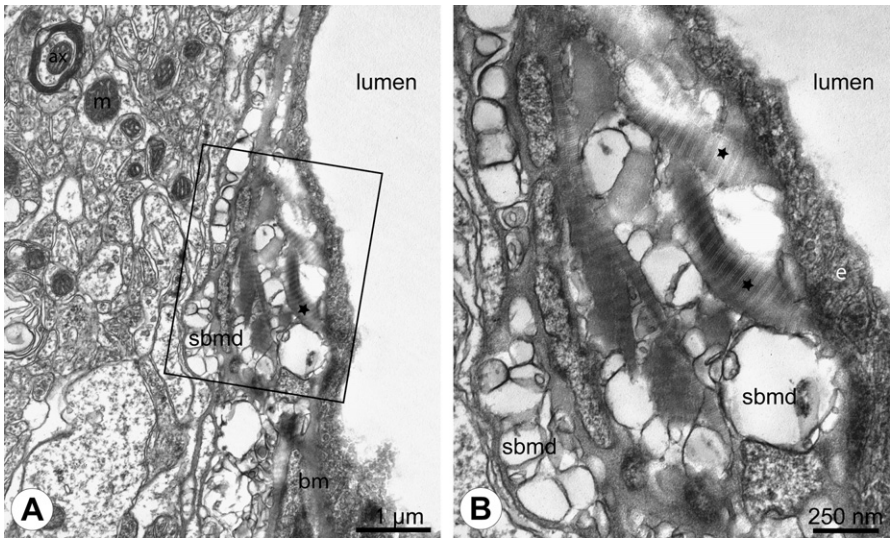

Fig. 6. Microvascular fibrosis in senescent hamster periaqueductal gray matter. (A) Spumiform basement membrane (bm) degeneration (sbmd) and collagen fibrils embedded within a split bm. (B) High magnification of the boxed area presented in (A). Collagen fibrils (asterisks) showing their characteristic periodicity are located intermingled with the vacuoles of sbmd. Abbreviations: ax, axon; m, mitochondrion.

hamster mesencephalic PAG appears mainly in a new and specific form of bm degeneration, which we have coined 'spumiform' bm degeneration (abbreviated sbmd) because of its spumiform (foamy-like) morphologic features. Our study identified a process of steadily increasing 'spumiform' degradation products within the lamina densa of the capillary bm. We hypothesized that this new form of bm degeneration most likely begins with local splitting or vacuolization of the lamina rara densa and over time gradually develops into a rim of spumiform degradation products positioned as a cuff around the capillary.

4.2. Are sbm and sbmd region-related?

There is ample evidence that BBB-aberrations are region-specific and are associated with major consequences for neural functioning (Goldman et al., 1992; Nandy et al., 1975; Shah and Mooradian, 1997; Threatt et al., 1971; Zlokovic, 2008, 2011). The present study has demonstrated that bm splitting was lower in the aged caudal brainstem compared with the aged PAG. In young animals the numbers of sbm were only minimal. Microvascular wall pathology of the spumiform type, however, proved to be highly region-specific. The mesencephalic PAG emerged as the most affected site, followed by the prf control area, in contrast to other caudal brainstem areas (NPRA, NTScom) where almost no sbmd was found. Sbmd was almost exclusively present in the PAG of aged hamsters and virtually not in PAG of young hamsters, suggesting a progressive process of capillary degeneration during aging of the PAG.

4.3. Specificity; are sbm and sbmd species-related?

Sbmd in the hamster PAG shares some characteristics with the membranous inclusions observed in the bm in the cerebral cortex (de Jong et al., 1990, p. 385; Fig. 3G) and in the dorsal lateral geniculate nucleus of the aged rat brain (Alba et al., 2004, p. 149; Fig. 5C and D), suggesting interspecies similarities. The latter study also showed sbm in a thickened basal lamina (Fig. 4; Alba et al., 2004, p. 148), but the authors did not comment on the presence of these splits. Characteristic forms of perivascular fibrosis like amorphous or structured collagen depositions, as shown in rat and human brain (De Jong et al., 1990; Farkas and Luiten, 2001; Farkas et al., 2001), were not observed in any of these areas in the hamster. In this context it has to be emphasized that in the sbmd's no ultrastructural characteristic of microvascular fibrosis or amyloid storage (De Jong et al., 1990) could ever be observed. The existence of sbmd at different locations in rat and hamster PAG warrants further investigation of this new type of capillary degeneration in other species, including senescent humans.

In that respect we performed a preliminary study on the location and morphologic configuration of sbm and sbmd in PAG capillaries of 2 aged human brains (71-year-old female and a 91-year-old male). Our ultrastructural analysis revealed increased numbers of bm thickening, increased vacuolization and irregular collagen fiber patterns in the bm and bm duplications, when compared with young adult human brains, similar to what has been described in the rat brain (Farkas and Luiten, 2001; Farkas et al., 2000a, 2001, 2006; Perlmutter and Chui, 1990) (Fig. 8A and B). Age-related microvascular degeneration in the human cerebral periventricular white matter was studied by Farkas et al. (2006) and by Perlmutter and Chui (1990) in Alzheimer's disease. It was suggested that bm thickening, bm splitting or vacuolization, and microvascular fibrosis are interrelated structural changes of the aging processes. Bm thickening could be the result of an increased production of bm components or a decreased breakdown of bm materials, such as collagen type IV, laminin, or heparan sulfate proteoglycan (Perlmutter and Chui, 1990). Sbmd with similar structural characteristics as observed in hamster PAG microvessels, could not yet be discerned convincingly in our human PAG tissue, because fine ultrastructural morphology was partially hampered by the combined negative effects of relatively long postmortem delay on nervous tissue (10 and 12 hours respectively) and necessity to use less adequate (immersion fixation) preservation techniques (Fig. 8A and B).

Based on the striking similarities in interrelated age-related microvascular changes, it is hypothesized that processes of sbmd with their unique characteristic features presented in this study are analogous with products of aging processes in rat and human bm as described above. The sbmd stages II and IV can be considered as final stages in the process of microvascular aging and as such share common structural features with the development of microfibrillar deposits in dilated bm as observed in rat and human (Farkas and Luiten, 2001; Farkas et al., 2000a, 2006; Perlmutter and Chui, 1990; Perlmutter et al., 1990). Reduction in PAG capillary bm integrity might have consequences for BBB function and therefore

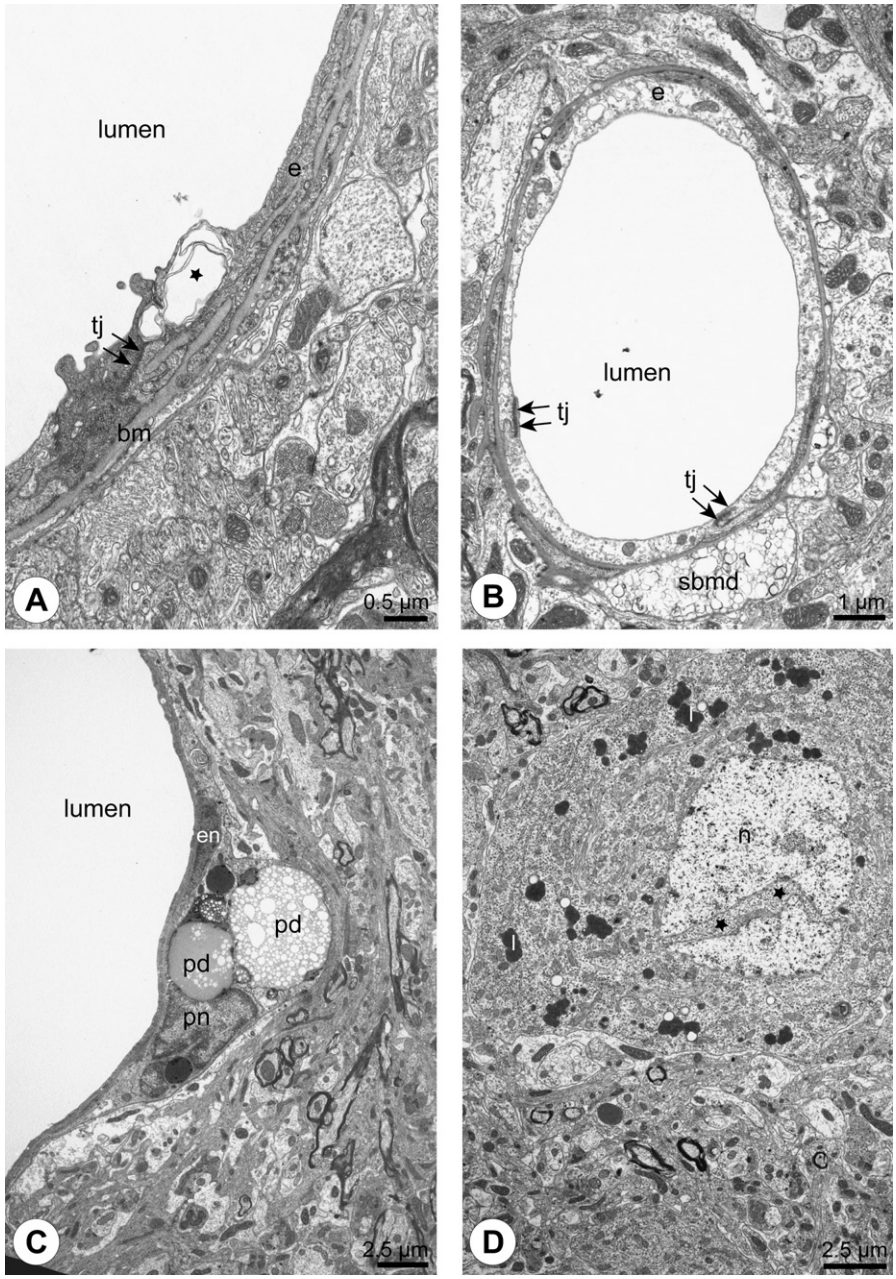

Fig. 7. Electron microscopic photomicrographs showing capillary degeneration and structural changes in periaqueductal gray matter (PAG). (A) Part of a capillary surrounded by a thin, endothelial cell layer (e) that forms a tight junction (tj) complex. The tj complex comprises a widened cap (asterisk) filled with membranes. The basement membrane (bm) is composed of several irregular layers running in parallel. (B) Edematous endothelial lining covering the lumen of a PAG capillary. The cytoplasm of the endothelial cell shows small vesicles and fine tubular elements. Tight junctions can be observed between overlapping cellular extensions (black arrows). Spumiform bm degeneration (sbmd) (stage III) covers a substantial part of the capillary wall. (C) Capillary basement membrane covered by a pericyte with typical age-related pericyte degeneration products (pd), large lysosome-like bodies that contain variable electron dense or lucent, autophagic inclusions. (D) PAG neuron. The nucleus (n) with irregular outline is surrounded by a rim of cytoplasm filled with clusters of lipofuscin granules (l). The nuclear membrane is invaginated (asterisks). Lipofuscin granules contain variable electron dense or to a lesser extent electron lucent accumulations.

might lead to impairment of autonomic and motor control functions as a result of the diminishing supply of oxygen, energy substrates, and nutrients (Brown and Thore, 2011; Zhong et al., 2008; Zlokovic, 2011).

4.4. Possible mechanisms of the development of perivascular cuffs

The PAG is localized directly around the Sylvian aqueduct, and might be vulnerable to hydrodynamic processes as a result of exposure to continuous passage of pulsating cerebrospinal fluid (CSF). Recent insights into hydrodynamics of CSF provide evidence that water, which constitutes 99% of CSF and interstitial fluid (ISF) bulk, are rapidly absorbed into microvessels adjacent to the central nervous system (Bulat and Klarica, 2011; Bulat et al., 2008). It appears that a process of water filtration across the walls of microvessels in the central nervous system is a key step in the production of ISF and CSF. Plasma osmolytes are retained, however, for generating capillary osmotic counter-pressure, which is essential for maintenance of ISF/CSF balance of water absorption into capillaries. The concentration of other macromolecular substances

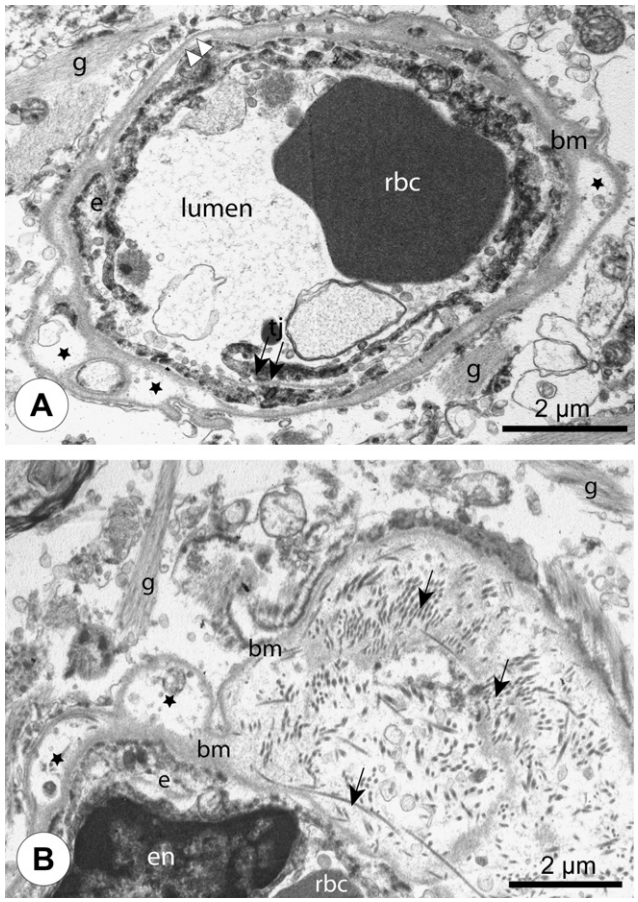

Fig. 8. Aberrated capillaries from periaqueductal gray matter (PAG) of a 91-year-old male subject (postmortem delay approximately 12 hours). (A) The capillary lumen contains part of an erythrocyte (rbc) and some laminar debris. Remains of a tight junction (tj) are indicated with black arrows. The basement membrane (bm) is locally thickened with split bm (white arrowheads), and contains extended electron lucent vacuolization, with membranous profiles (asterisks). The perivascular space shows gliosis (g) and artifactual space because of the delayed fixation. (B) Microvessel with split bm (asterisks), and extended electron lucent bm vacuolization with fibrosis (arrows). The perivascular space shows gliosis (g). In both (A) and (B) the perivascular space shows artifactual space because of postmortum tissue degeneration.

in the periventricular regions including PAG, depends on the rate of their removal into microvessels (for review, Bulat and Klarica, 2011). Microscopic data provide evidence that a decreased cerebral blood flow is associated with the accumulation of fibrous collagen in the microvascular walls (Farkas et al., 2000b). It can be argued that structural capillary wall changes are adaptations to altered perfusion and physiologic conditions because cerebral hypoperfusion has been found to have a deleterious effect on the neural tissue (Farkas et al., 2000a,b). Human aging leads to reduced cerebral blood and cerebrospinal fluid flows (Buckner et al., 2000; Grubb et al., 1977; Raichle, 1981; Stoquart-ElSankari et al., 2007; Yang et al., 2011).

Stoquart-ElSankari et al. (2007) demonstrated that the CSF stroke volumes are significantly reduced in the elderly (i.e., at aqueduct levels). Further, their results show a decrease of total cerebral blood flow, proportional aqueductal and cervical CSF pulsations-reduction as a result of arterial loss of pulsatility, and preserved intracerebral compliance with aging (Stoquart-ElSankari et al., 2007). Disturbances of CSF dynamics play a role in CSF mobility decline with aging especially in cases of unknown origin (Onen et al., 2005). Considering the above-mentioned studies and our recent findings, it is suggested that regional differences in the occurrence of the characteristic sbmd might be because of structural changes, related to hydrodynamics of ISF/CSF during aging. Because PAG capillaries are located close to the aqueduct, they might be significantly more vulnerable for capillary changes including sbmd than more caudally located brainstem structures.

4.5. Conclusion

In the present study we describe the development of a new category of microvascular degenerative changes in the midbrain periaqueductal gray matter in aging hamster which we designated as spumiform capillary bm degeneration (sbmd) which appeared to be highly region-specific. The nature and origin of sbmd is still unknown and we speculate that the membranous component of sbmd originates from collagen IV fibrils of the lamina densa. During aging these collagen fibrils might develop compartments that become gradually filled with high molecular translucent substances, that do not pass the bm lamina rara externa. Obviously, capturing of substances in the spumiform spaces might be the result of a selective permeability change in the different bm components.

Regarding the extent to which the flow rate and pressure of the cerebrospinal fluid (Jones et al., 1987; Kleine et al., 1993; May et al., 1990; Redzic et al., 2005; Reiber, 1994, 2003) play a role in the spumiform changes observed remains open for further research. We conclude that a reduction in PAG capillary wall integrity might have serious consequences for BBB function and impairment of autonomic and motor control functions of the PAG region.

Disclosure statement

The authors declare no conflicts of interest.

The protocols, surgical procedures, pre- and postoperative care, and handling and housing of the animals were in accordance with the ethical guidelines approved by the University Medical Center Groningen, University of Groningen (license number DEC 5142A). All efforts were made to minimize animal suffering and to reduce the number of animals used.

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